INTERNATIONAL JOURNAL OF PHARMACY AND ANALYTICAL RESEARCH

ISSN: 2320-2831

IJPAR |Vol.10 | Issue 2 | Apr - Jun -2021
Journal Home page: www.ijpar.com

Research Study Open Access

Analytical Method Development and Validation of Stability Indicating Assay Method for Estimation of Olmesartan Medoxomil and Hydrochlorothiazide in Dosage Form by RP-HPLC

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ABSTRACT

To develop a rapid, precise, accurate and sensitive reverse phase High Performance liquid chromatography method and Forced degradation studies for the estimation of Olmesartan medoxomil and Hydrochlorothiazide in dosage form. Method was performed on C18column (INERTSIL ODS 3V, 5µ, 150*4.6mm) using a mobile phase consisting of pH 3.0 phosphate buffer: Acetonitrile at a flow rate of 1.0 ml/min with UV detection at 262nm.drugs was subjected to UV degradation, Thermal Degradation, Humidity Degradation, Acid degradation, base degradation, Peroxide degradation and Neutral degradation respectively. This method is validated by using various validation parameters like system suitability, specificity, linearity, precision, accuracy, solution stability, filter interference and robustness. The retention time of Hydrochlorothiazide peak was at 5.242 and Olmesartan peak was at 8.480 respectively. The calibration curve of Hydrochlorothiazide was linear over the range of 15-90 µg/ml and Olmesartan medoxomil 24-144µg/ml respectively. Correlation coefficient was obtained at 0.999 for Olmesartan medoxomil and Hydrochlorothiazide respectively. The limits of detection of Hydrochlorothiazide and Olmesartan medoxomil were determined 1.18µg/ml and 0.754µg/ml. And limits of quantification of Hydrochlorothiazide and Olmesartan medoxomil were determined 3.58µg/ml and 2.28µg/ml respectively. The study show to facilitate the reverse phased liquid chromatography is sensitive and selective for detecting Hydrochlorothiazide, Olmesartan and its impurities using the gradient programme.

Keywords: Hydrochlorothiazide, Olmesartan medoxomil, RP-HPLC, Stability indicating method.

INTRODUCTION

Hydrochlorothiazide is with chemicals far-famed as 6-chloro-1, 1-dioxo-3, 4-dihydro-2H-1, 2, four benzothiadiazine-7-sulfonamide [Fig no: 1]. it's a White crystalline powder, Soluble in solvent, meagrely soluble in alcohol, terribly slightly soluble in water, dilute solutions of alkali hydroxides. It's utilized in the treatment of cardiovascular disease. Hydrochlorothiazide medical specialty action; it inhibits active chloride reabsorbing at the distal tube via the binary compound co-transporter, leading to Associate in Nursing raised within the excretion of binary compound and water. The medicine mechanism of Hydrochlorothiazide is a smaller amount well understood though it's going to mediate through its action on carbonous anhydrases within the swish muscle (or) through its action on the massive electrical phenomenon atomic number 20 activated potassium (KCa) channel, conjointly found within the swish muscle. This leads to a rise in metal excretion via the metal exchange mechanism.

Olmesartan Medoxomil is with chemicals referred to as 4-(2-hydroxypropan-2-yl)-2-propyl-1-(methyl)-1H-imidazole-

5-carboxylic acid[Fig no:2]. It's a White to off-white crystalline powder. Practically insoluble in water and meagrely soluble in wood spirit. Olmesartan is employed to treat high pressure level (hypertension). Lowering high pressure level helps forestall strokes, heart attacks, and urinary organ issues. Olmesartan belongs to a category of medication known as vaso constrictive receptor blockers (ARBs). It works by reposeful blood vessels so blood will flow additional simply. Angiotensin II is created from angiotensin during a reaction catalysed by vaso constrictive changing protein (ACE, kininase II). Angiotensin is that the principal pressure agent of the renin-angiotensin system, with effects that embody constriction, stimulation of synthesis and unleash of mineral corticoid, internal organ stimulation and urinary organ resorption of metal. Olmesartan blocks the agent effects of angiotensin by selection obstruction the binding of angiotensin to the AT1 receptor in tube-shaped structure swish muscle. Its action is, therefore, freelance of the pathways for angiotensin synthesis[1&2].

Extensive literature survey was distributed that disclosed that there's no work distributed particularly on High Performance liquid natural process technique and made degradation studies for the estimation of Olmesartan medoxomil and Hydrochlorothiazide in dose form[3-7]. Few reports on Spectrophotometric strategies also are accessible. With this background the target of the presented work is to develop an analytical technique development and validation of stability indicating assay technique for estimation of Olmesartan medoxomil and Hydrochlorthiazide in dose type by RP-HPLC[8-13].

MATERIALS AND METHODS

Instruments

Liquid chromatographic system from Waters HPLC equipped with PDA Detector Intertsil ODS 3V column (150*4.6mm), 5µm, Elma S 300H Ultra Sonicator was used.

Chemicals and Reagents

The working standard Olmesartan medoxomil and Hydrochlorthiazide Sample was collected from MYLAN Labs, R&D Center, and Hyderabad, India. Acetonitrile (HPLC Grade), Methanol (HPLC Grade), Potassium dihydrogen phosphate (KH_2PO4) (HPLC Grade), Orthophosphoric acid (AR Grade) was obtained from Merck Manufacturer, Mumbai, India.

Preparation of diluents

Mix Acetonitrile, methanol and water with in the ratio of 50:20:30 and keep it for sonication to degas the elucidation.

Preparation of mobile phase

Dissolve 1.36 g of potassium dihydrogen phosphate ($KH2PO_4$) in 1000mL Water, and adjust pH to 3.0$\pm$ 0.05 with diluted ortho-phosphoric acid solution. Filter through 0.45µm membrane filter (Millipore make).

Preparation of standard solution

Weigh accurately 80 mg of Olmesartan and 50 mg of Hydrochlorthiazide working standards /reference standards into a 100 ml volumetric flask, add 80 ml of diluents and keep it for sonication to permit the standard to dissolve for 1 min and dilute to volume with diluents. Pipette out 3 ml of the above standard stock solution into a 25 ml volumetric flask, dilute to volume with diluents & mix well. Filter the elucidation through 0.45 µm PVDF syringe filter.

Preparation of test solution

Take 10 Tablets average weight and transfer10 tablets into 250ml volumetric flask and add 10ml of water and shake the flask to disintegrate the tablets, Add 200 ml of diluents, sonicate for about 25mins with intermittent shaking, dilute to volume with diluent-1 and blend well, Centrifuge the portion of above solution at 5000 RPM in centrifuge tube with cap for about 10 minutes, Pipette 3 ml of the above clear supernatant into a 50 ml volumetric flask and dilute to volume with diluents, and blend well, Filter the above solution with 0.45 micron PVDF filter.

Chromatographic conditions

Quantitative HPLC method was performed on Waters HPLC system with PDA and UV detector. Empower software is employed together with a stainless steel column INERTSIL ODS 3V (150×4.6mm), 5µm. To develop an appropriate and robust HPLC method for the determination of Olmesartan medoxomil and Hydrochlorthiazide. Different mobile phases containing Water: Methanol (50:50), 10Mm pH 3.0 phosphate buffer: Methanol (50:50), 10Mm pH 3.0 phosphate buffer: Acetonitrile (60:40), at different flow rates. The mobile phase pH 3.0 phosphate buffer: Acetonitrile with a flow rate at rate of 1.0 ml/ min gave peaks of excellent resolution. The detection is performed at the wavelength 262nm.

VALIDATION

The developed technique was validating in conditions of linearity, specificity, precision, accuracy, robustness and ruggedness.

Linearity

Prepare a series of standard solutions (not less than 5 are recommended) within the range of 15µg/ml-75µg/ml of Hydrochlorthiazide standard and 24µg/ml-144µg/ml of Olmesartan Medoxomil standard injected. A plot of average peak area versus the concentration in µg/ml or mg/ml is complete and this correlation coefficient, y-intercept (const. of regression) and slope (coefficient of regression) of the regression curve were calculated. (Fig no 3 &4.Table no: 1)

Precision

The precision of the test process was evaluated Hydrochlorthiazide and Olmesartan Medoxomil by injecting

the six standard solutions. The Relative Standard Deviation of six injections was considered. (Table no: 2)

Specificity

Specificity is that the ability of a technique to discriminate between the analyte(s) of interest and other components that are present within the sample. A study of placebo interfering from excipients was conduct. Atomic weight of placebo taken as per the test method and placebo interference was conducted in duplicate.

Accuracy

To validate the test method can accurately quantify Hydrochlorthiazide and Olmesartan Medoxomil, prepare samples in 3 times for higher and lower levels, in triplicate for other levels by spiking Hydrochlorthiazide and Olmesartan Medoxomil, active material with equivalent amount of placebo and perform as per test procedure. Prepare samples at levels 50%, 100% and 150% of the target assay concentration i.e. 50% of the least strength initial concentration to 150% of the highest strength initial concentration level. (Table no: 3&4)

Robustness

Robustness of the method is performed by altering the chromatographic conditions such as changing the flow rate, change of temperature, Mobile phase composition and observed the variation of the results which should be within the acceptance criteria. (Table 5 & 6)

Forced degradation studies

Acid stressed degradation

For acid degradation studies, 1 ml of stock solution of Olmesartan medoxomil and Hydrochlorthiazide Treated with 0.05M(5ml) HCL solution for about 5min.

Base stressed degradation

For base degradation studies, 1 ml of stock solution of Olmesartan medoxomil and Hydrochlorthiazide Treated with 0.05M (5ml) NaoH solution was added separately, the solutions were kept for 10min.

Peroxide stressed degradation

For Peroxide degradation studies, 1 ml of stock solution of Olmesartan medoxomil and Hydrochlorthiazide Treated with 3%H202 (5ml) solution was added separately, the solutions were kept for 15min.

Neutral stressed degradation

For neutral degradation studies,1 ml of stock solution of Olmesartan medoxomil and Hydrochlorthiazide Treated with water(5ml) at 50C temperature for about 15min.

Humidity stressed degradation

For Humidity stressed degradation studies, Olmesartan medoxomil and Hydrochlorthiazide Exposed to humidity at 25C/90%RH for about 72hrs.

Thermal stressed degradation

For Thermal stressed degradation studies, Olmesartan medoxomil and Hydrochlorthiazide Exposed to heat at 50C temperature for about 30 min.

UV stressed degradation

For UV stressed degradation studies, Olmesartan medoxomil and Hydrochlorthiazide Exposed to UV light for about 73hrs or UV Light by keeping the beaker in UV Chamber for 7days**.** (Table no: 7) and (Fig no: 5-9)

RESULT AND DISCUSSION

The developed HPLC method for the estimation of Olmesartan medoxomil (40mg) and Hydrochlorthiazide (25mg) tablets has been developed and validated for determination in commercial dosage forms. The compound and its impurities were well separated by using gradient programme on a C18 column (INERTSIL ODS 3V, 5µ, 150*4.6mm) employing a mobile phase consisting of pH 3.0 phosphate buffer: Acetonitrile at a flow of 1.2 ml/min with UV detection at 262nm. The retention time of Hydrochlorthiazide peak was at 5.242 and Olmesartan peak was at 8.480. The procedure was validated for all compendial and non compendial parameters in accordance with ICH guidelines. The study showed that the reverse phased liquid chromatography is sensitive and selective for detecting Hydrochlorthiazide, Olmesartan and its impurities using the gradient programme.

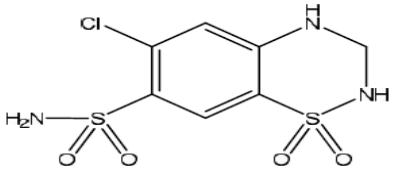

Fig No: 1 Structure of Hydrochlorthiazide

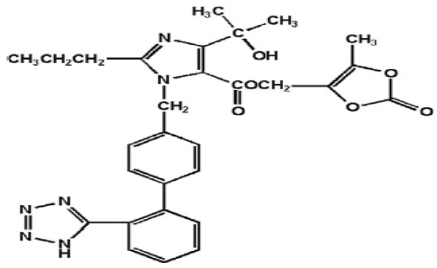

Fig No: 2 Structure of Olmesartan Medoxomil

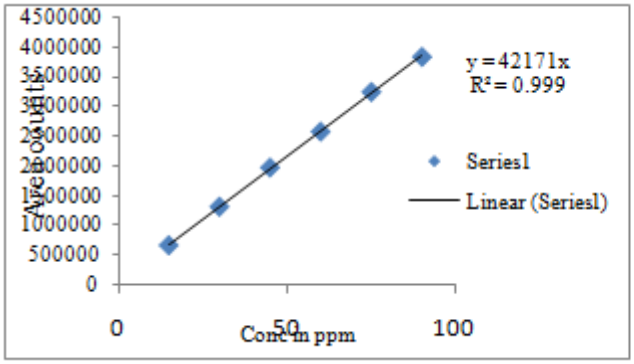

Fig No: 3 Linearity of Hydrochlorthiazide

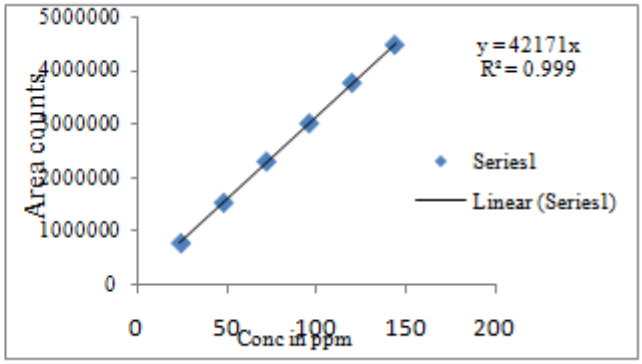

Fig No: 4 Linearity of Olmesartan Medoxomil

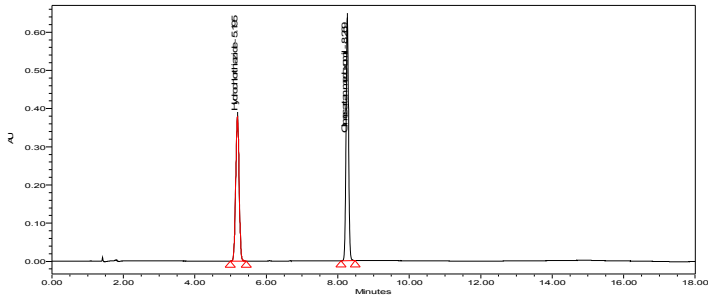

Fig No: 5chromatogram of Standard solution

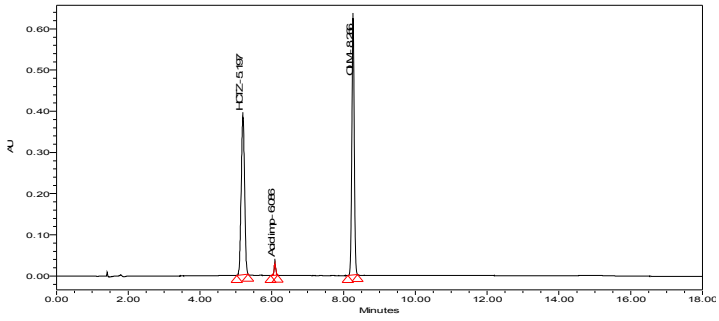

Fig No: 6Typical chromatogram of Acid stressed sample

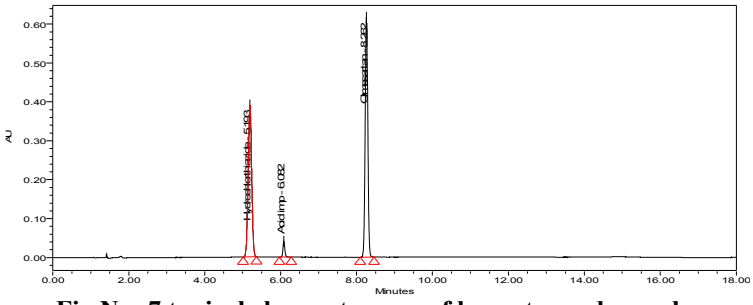

Fig No: 7 typical chromatogram of base stressed sample

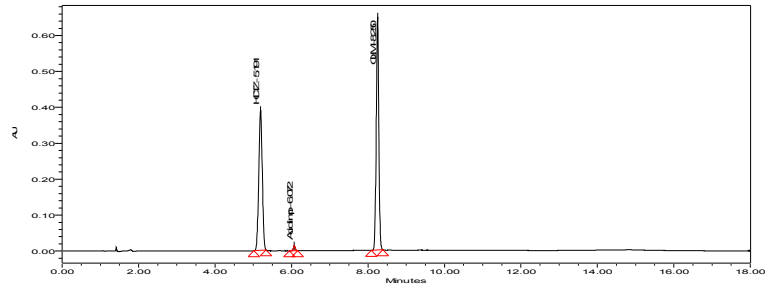

Fig No: 8chromatogram of Neutral stressed sample

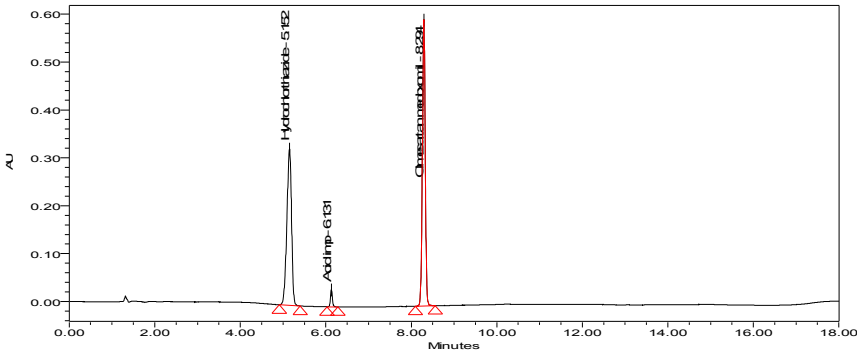

Fig No: 9Typical chromatogram of humidity stressed sample

Table no: 1 Linearity results of Olmesartan medoxomil and Hydrochlorthiazide

Standard concentration(µg/ml)		Area		Mean Area	
HCTZ	OLM	HCTZ	OLM	HCTZ	OLM
15	24	655529	779478	655490	779465
		655452	779452		
30	48	1301829	1533483	1308178.5	1531469
		1314528	1529456		
45	72	1957196	2301075	1951546	2305763
		1945896	2310452		
60	96	2558901	3014981	2558558	3014618
		2558215	3014256		
75	120	3223312	3770802	3223929	3768107
		3224547	3765412		
90	144	3810231	4480284	3810046	4480262
		3809862	4480241		
Regression	Hydrochlorthiazide=0.999		Olmesartan Medoxomil=0.999		

Table no: 2 Precision for Hydrochlorthiazide and Olmesartan

S.NO	Injection Number	Peak area for Hydrochlorthiazide	Peak area for Olmesartan Medoxomil	Acceptance Criteria
1	Standard 1	2540913	3095658	The %RSD for peak area of Hydrochlorthiazide and Olmesartan Medoxomil from six replicate injections of standard solution should not be more than 2.0
2	Standard 2	2509496	3091455	
3	Standard 3	2504314	3084434	
4	Standard 4	2507286	3086961	
5	Standard 5	2512208	3091031	
6	Standard 6	2521153	3096891	
Mean		2515895	3091072	
%RSD		0.5	0.2	

Table no: 3 Accuracy of Hydrochlorthiazide

%Level spiked	Sample No.	% Recovery	Mean % Recovery	% RSD
50	1	100.8	99.6	1.06
	2	98.8		
	3	99.2		
100	1	100.4	99.7	0.6
	2	99.2		
	3	99.6		
150	1	98.4	99.6	1.57
	2	99.5		
	3	101.5		

Table no: 4 accuracy of Olmesartan medoxomil

% Level spiked	Sample No.	% Recovery	Mean % Recovery	% RSD
50	1	99.8	99.5	0.2
	2	99.5		
	3	99.3		
100	1	99.8	99.7	0.2
	2	99.5		
	3	99.8		
150	2	100.2	100.2	0.6
	3	99.7		
	2	100.8		

Table no: 5 Results of Robustness -Hydrochlorothiazide

System Suitability Criterion Hydrochlorothiazide	Observed Value at Column Oven Temp.		Acceptance Criteria
	25°C	35°C.	
The USP Plate count	18459	13452	MT 2000
The Tailing factor from the chromatogram of Standard solution	0.9	0.9	≤ 1.0
% Relative standard deviation from five replicate injections of standard solution	0.1	0..2	≤ 2.0

Table no: 6 Results of Robustness -Olmesartan medoxomil

System Suitability Criterion Olmesartan	Observed Value at Column Oven Temp.		Acceptance Criteria
	25°C	35°C.	
The USP Plate count	25231	27444	MT 2000
The Tailing factor from the chromatogram of Standard solution	0.93	0.95	≤ 1.0
% Relative standard deviation from five replicate injections of standard solution	0.3	0.2	≤ 2.0

Table no: 7 Results of degradation studies for sample

Stress Conditions	% Degradation	Olmesartan medoxomil		HCTZ		Purity Flag	Acceptance Criteria
		Purity Angle	Purity Threshold	Purity Angle	Purity Threshold		
Treated with 0.05M(5ml) HCL solution for about	5.3	0.051	0.288	0.073	0.236	NO	

5min.							
Treated with 0.05M(5ml) NaoH solution for about 10min.	6	0.048	0.299	0.084	0.241	NO	The Purity angle should be less than Purity Threshold and no purity flag for both peaks
Treated with 3%H202(5ml) solution for about 15min.	12	0.065	0.283	0.107	0.236	NO	
Treated with water(5ml) at 50C temperature for about 15min	0.8	0.044	0.290	0.067	0.238	NO	
Exposed to humidity at 25C/90%RH for about 72hrs	9	0.053	0.259	0.074	0.231	NO	
Exposed to heat at 50C temperature for about 30	0.3	0.043	0.292	0.077	0.225	NO	
Exposed to UV light for about 73hrs	9	0.053	0.259	0.074	0.231	NO	

CONCLUSION

The developed HPLC method was validated by using various validation parameters like system suitability, specificity, linearity, precision, accuracy, solution stability, filter interference and robustness. All the validation parameters were found to be within the acceptance criteria. The HPLC method was found to be accurate, precise and reproducible. The technique can be useful for routine estimation of Olmesartan Medoxomil (40mg) and Hydrochlorthiazide (25mg) in pharmaceutical formulations.

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